SOME FUNDAMENTAL PROBLEMS OF

CELLULAR PHYSIOLOGY

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WILLIAM CHAUNCEY WILLIAMS, M.D.,
OF THE CLASS OF 1822, YALE MEDICAL SCHOOL
AND OF
WILLIAM COOK WILLIAMS, M.D.,
OF THE CLASS OF 1850, YALE MEDICAL SCHOOL

The Third
William Thompson Sedgwick Memorial Lecture

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SOME FUNDAMENTAL PROBLEMS OF *CELLULAR PHYSIOLOGY*

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SOME FUNDAMENTAL PROBLEMS OF CELLULAR PHYSIOLOGY

I SHALL not try to tell you how highly I value the privilege of speaking on this occasion. Nor shall I attempt to pay a tribute to him whose memory we recall today, for this has been done by others far better than I could hope to do it. But perhaps I may be permitted to say that in all the years of our personal acquaintance he seemed to me as one who worked in the spirit of Pasteur, with keen interest in both theoretical and practical researches, and in deep sympathy with every attempt to study the foundations upon which all applied science must rest. In consequence I feel it appropriate to dwell today upon some fundamental aspects of biology.

Two things of note in the recent development of biology may serve as my text. One is the increasing attention paid to the fundamental activities of the cell. The other is the growing

conviction that these activities may be largely determined by the permeability of the protoplasm. Hence the study of permeability has acquired especial importance not only in theoretical respects but in many practical problems, as, for example, the absorption of food, the application of drugs, and the physiology of excretion and secretion.

Let us consider for a moment certain activities which are common to all living things. All cells synthesize certain foods which exist in soluble form. What prevents these substances from diffusing out of the cell into the surrounding liquid, thereby depriving it of materials essential to its existence? Experiments have shown that the protoplasm is sufficiently impermeable to such materials to prevent serious loss and that it also possesses the power to exclude various substances, some of which are deleterious. At the same time it must admit raw materials out of which it manufactures food. Hence the protoplasm has selective permeability, permitting certain things to go in and out while barring the passage of others.

In order to understand how materials are absorbed and given out, we may consider first of all the structure of the cell. A typical plant cell consists of a cell wall (of non-living material) within which is a layer of living protoplasm, often so thin as to be little more than a delicate membrane. Within this is a large central vacuole filled with cell sap, and in many cases plastids (such as the chloroplasts) are present. A simple animal cell differs in several respects: of especial significance for our present purpose is the absence of the cell wall and of the central vacuole.

When a plant cell absorbs water the protoplasm expands and the cell wall is stretched. If water is withdrawn the cell wall shrinks; this shrinking soon stops but the protoplasm may continue to contract until it is drawn away from the wall. If it is allowed to reabsorb water it expands, stretching the cell wall with considerable force (strikingly manifested in growth, when mushrooms raise flagstones, or ferns push up through concrete sidewalks).

The manner in which this force is generated may be illustrated by tying a membrane to a

piece of glass tubing. If we select a membrane permeable to water but not to sugar, placing inside of it a solution of sugar and outside of it pure water, we find that as water is absorbed the level rises in the tube; if we endeavor to prevent this by means of a weighted piston placed in the tube, we find that considerable force is requisite. The pressure produced within the apparatus is called osmotic pressure.

It is supposed that pressure is produced in much the same way in the living cell. Here it is not the cell wall but the protoplasm (or its outer layer) which corresponds to the membrane in our experiment, and, like the membrane, it is permeable to water but is more or less impermeable to sugar and certain other substances. Such a membrane, permeable to some substances but not to others, is called semipermeable.

The discovery of these facts opened up a field of great interest but no great progress was made until quantitative methods began to be applied; as soon as this happened developments of the highest importance took place. The quantitative era may be said to begin with some observations

made in 1873 by Pfeffer, in his studies on the movements of irritable plant organs. He investigated particularly the behavior of certain stamens which, when touched by an insect, suddenly shorten, thereby depositing pollen upon the insect. The shortening is due to the giving out of water by the cells of the stamen; the water moves into the intercellular spaces, from which it is reabsorbed by the cells during the subsequent process of recovery. This reabsorption stretches the cell walls, thereby lengthening the stamen. To accomplish this a considerable force is required, which Pfeffer estimated at from two to four atmospheres.

At that time no one could explain how so much pressure was produced. Pfeffer sought to throw light on the question by constructing a model which would act as nearly as possible like the living cell. He prepared an unglazed porcelain cup in the pores of which he produced a deposit of copper ferrocyanide (after the method of Traube): this was closed by a stopper in which a manometer was fixed. The membrane of copper ferrocyanide is permeable to water but

not to sugar: if a sugar solution is placed inside and the cup is then immersed in water the latter is absorbed and the manometer registers an increase of pressure.

Pfeffer regarded the copper ferrocyanide membrane as analogous to the protoplasmic surface of the cell, the sugar solution in the interior corresponding to the solution of sugars, and other food substances, contained in the central vacuole.

The question with which Pfeffer started out was apparently answered. Since the artificial model could produce as much pressure as the plant cell the mechanism might be regarded as satisfactorily explained. Pfeffer, however, went further, investigating the effects of concentration and temperature and coming to the conclusion that the pressure is directly proportional to the concentration, and that it increases about 1/273 when the temperature rises 1° C.

In the meantime van't Hoff became interested in the attraction between water and other substances, a problem which is intimately connected with osmosis. One day as he left his laboratory,

thinking deeply on this question, he met de Vries, who told him the results of Pfeffer's observations. Seldom has an interchange of scientific ideas been more fruitful than this historic interview. It is a classic example of cross fertilization among the sciences. Not only did it initiate a new era in physical chemistry, but it was destined to be of fundamental importance to biology.

Pfeffer's results led van't Hoff to the revolutionary notion that molecules of dissolved substances obey the laws of gases. At that time it seemed possible that the application of these laws might clear up at one stroke most of the difficulties which had made the study of solutions so puzzling. This idea had far-reaching results.

An important difficulty remained to be accounted for. Inorganic salts failed to obey the general law which governs the osmotic behavior of sugar and many organic substances. de Vries had observed this in experiments on plant cells and had expressed the deviations from the law by means of the so-called "isotonic coefficients."

There was, however, no clue to the cause of this deviation. In general the inorganic salts were found to give too much osmotic pressure.

The difficulty seemed to be cleared up by the theory of Arrhenius, which states that all the exceptional substances have the power to conduct the electric current in solution and that they owe this property to the splitting of their molecules into electrically charged ions. This explains why they have abnormally high osmotic pressures, for each ion produces as much osmotic pressure as the molecule itself.

There remained the problem of finding a generally useful method of measuring the osmotic pressure of living cells. de Vries took this up. Instead of allowing the cell to absorb water, as in Pfeffer's experiments, he employed the opposite method, withdrawing water from the cell. If the cell absorbs water with a certain force we may oppose to it an equal force so that no absorption takes place. If we increase the opposing force, water will flow out of the cell, and this may cause the protoplasm to shrink away from the cell wall.

If a 5 per cent sugar solution extracts a little water from the cell its osmotic pressure must be a little greater than that of the solution in the cell. Since the osmotic pressure of a 5 per cent sugar solution can be determined, the osmotic pressure within the cell can be estimated.

In this way de Vries not only measured the osmotic pressure of a large number of plant cells but he performed the remarkable feat of determining by means of such cells the molecular weight of a compound which baffled the best efforts of chemists. This substance is a sugar, raffinose, for which three different formulae were proposed. Although the chemists were unable to decide which formula was correct it was for de Vries a simple matter. The three formulae gave the molecular weights 396, 594, and 1188. If raffinose has the same molecular weight as cane sugar (342) a 5 per cent solution will extract water from the plant cell to the same extent as a 5 per cent solution of cane sugar. But if its molecular weight is only half as great the 5 per cent solution will contain twice as many molecules and its power to draw out

water will be twice as great. This follows from the fact that the osmotic pressure is proportional to the number of molecules present (since sugar molecules do not split up in solution, as do molecules of inorganic salts). de Vries found that a 5 per cent solution of raffinose had less osmotic pressure than a 5 per cent solution of sugar, hence it must contain fewer molecules and its molecular weight must be greater than that of cane sugar. His calculations gave a molecular weight of 596, which differs by only a third of 1 per cent from one of the molecular weights (594) proposed by the chemists.

Much of the work of de Vries in this field preceded the investigations of van't Hoff and Arrhenius and helped to lay the foundation for them. This was particularly the case with regard to the substances which produce unexpectedly great osmotic pressure. de Vries rendered an indispensable service by calling attention to these cases and by carefully measuring the pressure produced, thus paving the way for the work of Arrhenius.

About this time important studies on the

osmotic pressure of animal cells were made by Hamburger, Grüns, Hedin, and others.

There remained another problem of great interest, namely, the nature of the semipermeable surface of the cell. It is evident that this determines what substances can enter the cell, and that on its composition the whole metabolism of the cell may depend. Pfeffer and others had suggested that it consists of protein, but the first systematic investigation of its nature was made by Overton. It had been suggested by Quincke that the outer layer of the cell consists of a film of oil: Overton came to a similar conclusion, substituting for "oil" the term "lipoid," which includes such substances as lecithin and cholesterin.

Overton concluded that no substance can enter the cell unless it is soluble in lipoid. The most important evidence which he brought forward may be summarized as follows:

1. Salts (which are as a rule insoluble in lipoid) are unable to enter the cell.

2. Dyes which are insoluble in lipoid do not

enter the cell, while lipoid-soluble dyes penetrate readily.

3. Organic substances (including such anesthetics as ether and chloroform) penetrate and affect the cell the more readily the more soluble they are in lipoid.

Overton's hypothesis has been a very valuable stimulus to investigation but it has become increasingly evident that satisfactory progress is impossible without more accurate measurements and a number of attempts have been made to provide suitable methods. It is evident that the best procedure is to expose the cell for a suitable time to a solution and then to analyze the cell-contents to discover what has penetrated. This is beset by difficulties which investigators have sought to overcome in various ways.

Some have made analyses of tissues, but it is evident that these must include too much intercellular material to be satisfactory. Analysis of the solution bathing the tissue in order to determine what is absorbed, is open to the objection that substances collect on the surfaces of cells, as well as in the cell walls and in the spaces be-

tween them, so that it is impossible to say what actually penetrates the protoplasm. Others have sought to analyze the cell sap. Plant cells are most favorable for this purpose, since, as a rule, they contain vacuoles filled with sap. In general the method has been to crush the tissues and express the sap, but this procedure involves many possibilities of error, such as contamination of the cell sap by substances present in the cell walls or intercellular spaces and chemical reaction between the cell sap and the crushed protoplasm or the cell walls. (The degree of pressure used in expressing has a marked influence on the concentration of the sap.) The investigation of blood and other body fluids is open to the objection that we do not know to what extent substances penetrate between the cells in reaching these fluids. In many of these cases penetration seems to present very special features.

The taking up of dyes has been extensively investigated but this method is beset by many pitfalls. To a great extent the coloration of the cell by a dye shows the extent to which the dye can combine with the substances within the cell

rather than the permeability. Some cells contain substances which combine with the dye so that it becomes far more concentrated within the cell than in the external solution. Unless the cell has this power it often fails to appear colored even though it may contain the dye in the same concentration in which it exists outside. In such cases it may sometimes be detected by plasmolyzing the cell and thus concentrating the dye. A further complication is that a cell may appear to have taken the dye into its interior when in reality only the surface or the cell wall is stained. There are many other difficulties, which need not be discussed here, such as toxic action and changes in the dye (including decolorization as it enters the cell).

In some cases the penetration of acids and alkalies has been studied by means of organisms containing natural indicators or by introducing indicators into the cell. Use has also been made of the fact that the penetrating substance may cause a visible precipitate within the cell; this is especially the case with alkaloids. Furthermore the absorption of calcium has been detected by

observing the formation of crystals of calcium oxalate within the cell. It is evident, however, that these methods have but limited application, and that in many cases they are open to the objection that the penetrating substance injures the cell.

The penetration of a substance may sometimes be demonstrated by observing its effect upon metabolism, but this method is inadequate from a quantitative standpoint. Some investigators contend that substances may produce effects on metabolism by their action at the surface, without actually penetrating the cell.

Owing to these difficulties a number of indirect methods have been employed. Of these plasmolysis has been the most popular. The principle is very simple. If to the fluid surrounding the cell a substance be added in sufficient concentration it will cause the withdrawal of water and shrinkage of the protoplasm, but if this substance subsequently penetrates, the osmotic pressure inside will increase and water will be taken up. When the concentration of the substance inside equals that outside, the cell will contain ap-

proximately as much water as before the substance was added. The cell in consequence recovers its normal appearance. The time required for such recovery is commonly regarded as an approximate index of the rate of penetration of the substance in question.

Even under the most favorable conditions this method is more or less injurious. When the protoplasm is torn away from the wall of a plant cell its surface is usually altered and is often torn so that detached bits of protoplasm remain adhering to the wall. Even if the cell subsequently recovers after such treatment we cannot be sure that during the experiment it was entirely normal.

Another method is by measuring electrical resistance. In earlier experiments it appeared possible to measure the permeability of normal cells to ions by this method. In measuring the electrical resistance of the marine plant *Laminaria* by means of an alternating current it was assumed that the current passed in part through the protoplasm and in part through the spaces between the protoplasmic masses. Hence by making al-

lowance for the latter part it seemed feasible to determine to what extent ions entered the protoplasm and carried the current through it.

In the case of *Laminaria* similar results are obtained whether we use direct or alternating current. But recent experiments on very large (multinucleate) cells, carried out in my laboratory by Mr. Blinks, indicate that the resistance of protoplasm to a direct current is very much greater than to an alternating current. It therefore seems probable that the surface of the cell acts as a condenser and transmits an alternating current without actual passage of ions. But measurements of resistance to an alternating current may nevertheless be very useful in detecting changes in permeability to ions. If the normal cell exhibits a high resistance and if this is lessened by a toxic agent, it shows that its permeability to ions has been increased. As a matter of fact determinations of electrical resistance by the means of alternating current show a progressive increase of permeability during the process of death and this is borne out by a variety of other evidence, such as the increased permeability to

dyes (and other substances) which is observed in dead or dying cells.

An advantage of the electrical method is that it gives us time curves of the process of death which can be analyzed mathematically and which enable us to make quantitative predictions regarding the behavior of protoplasm under various conditions. It may also enable us to place upon a quantitative basis such fundamental conceptions as normal vitality, injury, recovery, and death, which have hitherto been vaguely defined.

We find, for example, that when the normal electrical resistance has been ascertained it is a simple matter to test material as it comes into the laboratory and to tell whether it is in normal condition or not. If it is not normal we can determine just how far it deviates from normality and predict with considerable accuracy how long it will live.

As an illustration of the method we may consider what happens when the marine plant, *Laminaria*, is taken out of its normal environment of sea water and placed in a solution of

pure sodium chloride. We find that it is at once injured, and if the exposure be sufficiently prolonged it is killed. During the whole time of exposure to the solution of sodium chloride its electrical resistance (measured with an alternating current) falls steadily until the death-point is eventually reached; after this there is no further change. A study of the time curve of this process shows that it corresponds to a monomolecular reaction (slightly inhibited at the start). This may be expressed in the form of an equation which can be utilized to predict the curve of death under various conditions. We find that in testing these predictions we must ascertain when the death process reaches a definite stage (i.e., when it is one-fourth or one-half completed). This can be determined experimentally with a satisfactory degree of accuracy.

We can therefore follow the process of death in somewhat the same manner that we follow the progress of a chemical reaction *in vitro*; in both cases we obtain curves which may be subjected to mathematical analysis, from which we may draw conclusions regarding the nature of

the process. This method has been fruitful in chemistry and it is possible that it may prove equally so in biology.

Studies undertaken from this point of view lead us to look upon the death process as one which is always going on, even in a normal, actively growing cell. In other words we regard the death process as a normal part of the life process, producing no disturbance unless unduly accelerated by an injurious agent which upsets the normal balance and causes injury so that the life process comes to a standstill.

The process of death which occurs in a solution of sodium chloride may be checked by adding a little calcium chloride to the solution. In this case we speak of antagonism between sodium and calcium. When the calcium is added in the proper proportion the fall of resistance is very slow and the tissue lives for a long time. Any deviation from this optimum proportion hastens death.

When the plant is injured and the resistance falls, we may consider that the loss of resistance gives a measure of the amount of injury. This

enables us to place the study of injury upon a quantitative basis. As the result of this we are able to formulate a definite conception of the mechanism of recovery. We find that if injury in a solution of sodium chloride amounts to five per cent, the tissue recovers its normal resistance when replaced in sea water. But if the injury amounts to twenty-five per cent, recovery is incomplete; instead of returning to the normal the resistance rises to only ninety per cent of the normal. The greater the injury the less complete the recovery. When injury amounts to ninety per cent there is no recovery.

This is of especial interest, since in physiological literature it seems to be generally assumed that when recovery occurs it is always complete, or practically so, as if it obeyed an "all or none" law. But it is evident that partial recovery may be easily overlooked unless accurate measurements can be made. This fact may serve to illustrate the importance of quantitative methods in the study of these fundamental problems.

The significance of this method is further

shown by the fact that it has led to the development of equations which enable us to predict with a satisfactory degree of accuracy the recovery curves which are observed under a great variety of conditions.

As the result of these investigations we are led to look upon recovery in a somewhat different fashion from that which is customary. While recovery is usually regarded as due to the reversal of the reaction which produces injury, the conception here developed is fundamentally different. It assumes that the reactions involved are irreversible (or practically so) and that injury and recovery differ only in the relative speed at which certain processes take place.

These experiments lead to the view that life depends upon a series of reactions which normally proceed at rates bearing a definite relation to each other. If this is true it is clear that a disturbance of these rate-relations may have a profound effect upon the organism, and may produce such diverse phenomena as stimulation, development, injury, and death. Such a disturbance might be produced by changes of

temperature (if the temperature coefficients of the reactions differ) or by chemical agents. The same result might be brought about by physical means, especially where structural changes occur which alter the permeability of the plasma membrane or of internal structures (such as the nucleus and plastids) in such a way as to bring together substances which do not normally react.

The results obtained lead to a very simple conception of life processes, namely that they consist of a series of consecutive reactions. By varying the speed of the different members of the chain we can, without introducing any new substance, profoundly influence the course of events. In this way the effects of the most diverse stimuli on such processes as growth, differentiation, reproduction, and irritable response might be accounted for.

The results obtained by the electrical method have been confirmed by a variety of other methods, as, for example, by the passage of substances through diaphragms of living tissue. In this case diffusion takes place principally between the cells, but as permeability increases it

takes place to an increasing degree through the protoplasm and this increase can be measured.

In order to determine what substances enter under normal conditions we require accurate determinations of actual penetration by direct analysis of the contents of the cell. I have been so fortunate as to find plants in which this is possible. These have large multinucleate cells from which the sap can be squeezed out without danger of contamination and without any of the handicaps which rendered previous attempts, made with cells of ordinary size, unsatisfactory.

The fresh water plant *Nitella* produces multinucleate cells up to six inches in length and a thirty-second of an inch in diameter. Within the cellulose wall is a delicate layer of protoplasm containing nuclei and chloroplasts. The chloroplasts have a regular arrangement which is disturbed if the cell is slightly injured; at the same time the chloroplasts become more opaque. These are valuable criteria of normal condition.

Within this layer of protoplasm is the large central vacuole filled with sap in which float globules of protein, the whole being as a rule

in constant motion, flowing spirally up the cell in one direction and down in the other, so that the motion is opposite on the two sides.

By cutting one end of the cell and applying gentle pressure the sap can be squeezed out, free from protoplasm or chloroplasts. In making experiments the cell is placed in a solution of the substance whose penetration we are studying, removed after a suitable exposure, rinsed, and dried on the surface by means of filter paper. The sap may then be squeezed out without danger of contamination, since it is not allowed to come in contact with the surface except for an instant in the immediate neighborhood of the cut, and even this contact may be avoided by piercing the cell with a fine glass capillary and collecting the sap by means of gentle suction or pressure.

Analysis of the sap reveals a most interesting series of facts. Nearly all of the substances contained in it exist at a much higher concentration than is found outside. There must be a mechanism which traps these substances and causes them to accumulate. In order to discover what this

mechanism is, it is necesary to experiment with a substance which accumulates and to analyze the process step by step.

The first of such quantitative investigations on *Nitella* were made by Miss Irwin, using a basic dye, brilliant cresyl blue. The technique is very simple: after suitable exposure of the cell to the dye the sap is drawn up into capillary glass tubes whose color is compared with that of similar tubes filled with dye of known concentration. The accuracy of the method is satisfactory, since it is possible to distinguish between concentrations of .000017 and .000024 molar. This procedure avoids all the difficulties previously mentioned in connection with experiments on dyes.

The penetration of the dye follows the time curve of a reversible monomolecular reaction. It is possible that the dye combines with some constituent of the cell, giving a reversible reaction which appears to be monomolecular because the concentration of the uncombined dye is constant.

The idea of chemical combination is sup-

ported by the fact that the temperature coefficient is high.

On the other hand it may be assumed that the dye enters as a free base which dissociates more in the acid cell sap than in the more alkaline external solution and so accumulates in the fashion discussed later on in connection with the penetration of hydrogen sulphide into *Valonia.*

Of especial interest is the fact that as the alkalinity of the external dye solution increases the rate of penetration increases and the excess of concentration of the dye inside over that outside also increases. This indicates that the penetrating substance is the free base of the dye whose concentration would wax with increasing alkalinity. This idea is also in harmony with the experiments on exosmosis which show that when a stained cell is placed in a solution destitute of dye, the dye comes out more rapidly the greater the acidity of the external solution. We might explain this by saying that as the free base comes out of the cell the concentration gradient is greatly increased if the free base is at once changed into another form; this would

take place the more readily as the external solution becomes more acid.

While these studies were being made on *Nitella* The Rockefeller Institute for Medical Research very generously made it possible to carry on investigations on the marine alga *Valonia*. This forms large multinucleate cells, shaped somewhat like a balloon, containing up to 10 cubic centimeters of sap. The sap is destitute of the motion observed in *Nitella;* it is surrounded by a delicate layer of protoplasm containing chloroplasts and numerous nuclei: this is surrounded by a cellulose wall.

The use of large cells enables us to find out what goes on inside to a greater extent than was previously possible. As a result of these studies I have formed quite different conceptions of the behavior of the interior of the cell from those which I previously entertained. It has become apparent that we must now restate our problems and begin a fresh attack upon the whole subject. Although it is too soon to try to picture these new conceptions with any degree of complete-

ness a brief outline of some of them may not be amiss.

Consider first of all the astonishing difference which may exist between the inside and the outside of the cell. *Valonia macrophysa*, which grows in sea water, excludes certain substances absolutely as long as it remains in a normal condition; this is the case, for example, with magnesium and with sulphate. If it admits calcium at all it is only in traces. Sodium is admitted but its concentration remains far below that in the sea water. On the other hand, potassium is stored up within the cell in much greater concentration than is found in sea water. It is evident that the cell possesses a trapping mechanism for accumulating certain substances and also a means of excluding certain other substances either wholly or in part.

As a consequence the inner and outer surfaces of the protoplasm may be in contact with very different solutions. Is it necessary for the life of the cell that they should be different? What will happen if they are made identical? To answer this question sap was extracted and living

cells were placed in it, thus bringing the inner and outer surfaces of the protoplasm in contact with identical solutions (since the sap can be extracted without being altered). Most of these cells lived but a short time (as a rule less than a week), while under the same conditions in sea water the majority live for several months. This does not seem to be due to bacterial action for the cells died promptly in sap which had been boiled and filtered.

Normally there is considerable difference between the acidity of the sap and the sea water (the pH values of sap and sea water are 5.8 and 8.2 respectively). When cells are placed in their own sap this difference is abolished. Is this responsible for their rapid death? To answer this question the acidity of the external sap was varied; the results showed that acidity is not the primary factor involved although cells die much more quickly at pH 5.8 than at 8.2.

The important factor might conceivably be the maintenance of differences in electric potential between the inside and outside of the protoplasmic layer but this does not seem to be the

case, as measurements show that practically no such difference exists.

The primary factor seems to be a lack of certain elements in the external medium which are needed to make a "balanced solution." It is evident that the sap is not a balanced solution in the ordinary sense and this raises the question whether balanced solutions are in general necessary for the interior of the cell.

There is some ground for believing that the maintenance of differences between the interior and exterior of the cell is essential for carrying on vital processes in general.

The fact that juices from the interior of the cell may be toxic when applied to the exterior may find a parallel in certain observations on animals. Many investigators believe that when tissues are burned or crushed, toxic substances are set free. It may be that these substances are due to reactions which never take place except as the result of injury, but it is possible that some of these substances are normally present in the interior of the cell, in which case we should have an analogy to what is observed in *Valonia*.

The analysis of the sap of *Valonia macrophysa*, which was collected at Bermuda, agrees fairly well in general with that of the sap of *V. utricularis* collected at Naples. It is therefore very surprising to find that another species, *V. ventricosa*, collected at Bermuda, differs *in toto* from *V. macrophysa* in the composition of its sap. This is shown by Table I. It is evident that *V. ventricosa* has a sap whose composition is much like that of sea water.

TABLE I

Molecular Composition Expressed as Per Cent of Halide (Cl+Br).

	Bermuda sea water	Sap of *Valonia macrophysa*	Sap of *Valonia ventricosa*
Cl + Br . .	100.00	100.00	100.00
Na	85.87	15.08	92.80
K	2.15	86.24	2.58
Ca	2.05	0.288	1.36
Mg	9.74	Trace?	2.49
SO_4 . . .	6.26	Trace?	Trace?
Organic matter parts per thousand . .		1.433	2.09

These striking differences between two species of *Valonia* raise the question whether it is justifiable to assume, as is often done, that close relationship involves similarity in chemical composition and metabolic processes. The implications of this question are far-reaching. If it is possible for nearly allied forms to differ so profoundly, it is evident that we have as yet no satisfactory conception of the nature of the variables involved in differentiation or in the development of specific characters.

These differences raise another question of considerable importance, whether sodium or potassium predominates in the sap of plant or animal cells. For the study of this question it is desirable to employ cells whose sap can be obtained without alteration, as in the case of *Valonia* and *Nitella*. *Nitella* from Woods Hole shows approximately equal amounts of sodium and potassium; in a species collected near Cambridge there is a little more potassium than sodium. Hoagland and Davis state that in *Nitella clavata*, taken from pond water, the proportion of potassium to sodium in the sap is as

5.43 to 1. In the case of large cells in tap water, however, they report that sodium and potassium were more nearly equal, and in small cells in culture solution sodium predominated over potassium.

In these cases the sap could be extracted without contamination or alteration. Where this is not possible we cannot be sure of its composition. Ordinary ash analyses, made without extracting sap, do not tell us whether substances are present in soluble or insoluble form, whether they are located within the cell or in intercellular spaces. But such analyses may nevertheless give us some idea of the general situation.

Ash analyses show a decided predominance of potassium over sodium throughout the flowering plants and mosses. In the case of marine algae such analyses frequently show a preponderance of sodium over potassium, but this might be due to sea salts held in the cell walls or adhering to the surfaces of the plant.

The analyses of animals are less consistent than those of flowering plants but in the majority of cases potassium seems to predominate over

sodium. Erythrocytes are of special interest. In some cases they contain little or no sodium and there is reason to believe that the large amount of potassium which is present exists in solution as an inorganic salt. On the other hand there are species whose erythrocytes contain mostly sodium with very little potassium.

It is therefore apparent that we are not warranted in concluding that potassium invariably predominates over sodium in the sap of living cells.

It may be noted that by adding ammonium chloride to the sea water it is possible to substitute ammonium to a certain extent for the sodium and potassium of the sap without apparently injuring the cell.

Another interesting problem is suggested by the fact that the dissimilarity in composition of the two Valonias bring about a marked difference in their behavior in that *V. macrophysa* sinks in sea water while *V. ventricosa* floats. It may be said in this connection that it would seem that the flotation of living cells may be brought about

in a variety of ways, and that the subject deserves more careful investigation.

Another problem which has been attacked by the use of these large cells is whether protoplasm is permeable to ions. This question, answered in different ways by opposing schools, has become a center of controversy. Each side has assembled an imposing array of facts on every point of importance but the evidence remains conflicting and the interpretation doubtful. It is evident that the most satisfactory way of attacking the problem is to study the penetration of substances which are partly ionized and to determine by direct analysis whether the concentration of such a substance inside the cell corresponds to the ionized or to the non-ionized part of the substance in the external solution.

A suitable material for this purpose is hydrogen sulphide, which penetrates readily, is easily measured, and is only slightly toxic at low concentrations.

The experiments show that when a living cell of *Valonia* is allowed to take up hydrogen sulphide until the sap comes into equilibrium with

the sea water in respect to this gas, the concentration inside is always less than outside (except in quite acid solutions), and as the alkalinity of the external solution increases, and the hydrogen sulphide becomes more completely ionized, its concentration in the sap becomes less. This is shown in Fig. 1, in which the concentration of total hydrogen sulphide in the sap is designated by the symbol (○) and the concentration of undissociated hydrogen sulphide in the sea water is designated by the symbol (□) when calculated from the dissociation constant, by the symbol (△) when determined by the method of vapor tension, and by the symbol (×) when measured by the rate of evaporation.

Not only the inside concentration but also the rate of penetration corresponds closely to the concentration of undissociated substance outside. This indicates that under normal conditions hydrogen sulphide cannot enter the cell very rapidly except in the form of undissociated molecules and that it does not dissociate to any great extent after entering the cell. Precisely

the same conclusion is reached as the result of studies on the penetration of carbon dioxide.

It is true that, as far as final concentrations are concerned, we might expect a similar result

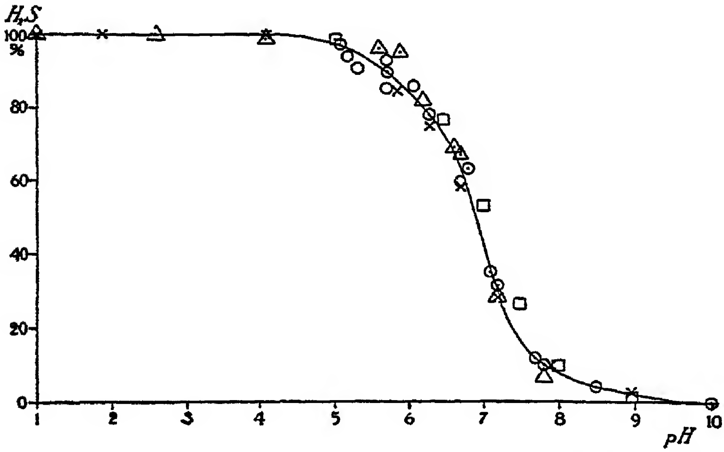

Fig. 1 demonstrates that the total sulphide in the sap corresponds with the undissociated H_2S in the external solution. Ordinates represent undissociated H_2S in the case of sea water and total sulphide ($H_2S + HS' + S''$) in the case of sap. Abscissae represent pH values. The concentration of total sulphide ($H_2S + HS' + S''$) in the cell sap (○) is expressed as per cent of the total sulphide in the outside solution. The values for the concentration of undissociated H_2S in the sea water as calculated from the dissociation constant (□) and as determined from the vapor tension (△) and from the rate of evaporation (×) at various pH values of the external solution are expressed as per cent of the corresponding values in the range pH 1 to 3 where all the H_2S is regarded as undissociated. Each point represents one determination.

if the ions of these substances entered and if a Donnan equilibrium were set up in a certain way, but in that case we might expect to find a different behavior in respect to rate of penetration. We may expect the rate of penetration to rise as the concentration of the penetrating substance increases. If it is the undissociated molecules which enter, this is what we actually observe, for as the acidity of the sea water increases the concentration of undissociated molecules and the rate of penetration likewise increase. If, on the other hand, we assume that it is the ions which enter, we should be obliged to conclude that as their concentration increases (with increasing alkalinity of the sea water) the rate of penetration falls off.

It is evident that (except when there is an ionic exchange in opposite directions) a cation cannot pass through the surface without a corresponding anion, so that in general a cation can pass only when it strikes the surface at the same time as an anion. Hence in many cases a cation must strike the surface without entering but in the case of an undissociated molecule every one

which strikes the surface may penetrate providing there is no other hindrance except the electrical forces. On this basis we should expect undissociated molecules to enter more rapidly than ions.

In this connection it may be noted that the work of Loeb, Harvey, Crozier, Haas, Jacobs, M. M. Brooks, Smith, Clowes, and Beerman (on various weak acids) indicates that undissociated molecules penetrate, although the methods employed do not enable us to decide positively whether ions enter or not. Those who have concluded that ions cannot penetrate have done so on purely theoretical grounds or as the result of indirect evidence. This also applies to a large extent in cases where the opposite conclusion has been reached.

If it should turn out to be generally true that ions are unable to penetrate except very slowly how shall we regard the evidence for the contrary view? This evidence rests chiefly on experiments with plasmolysis and electrical conductivity. It is found that many cells recover after plasmolysis when left in the plasmolyzing

salt solutions (provided they are not too concentrated). Since these salts are largely ionized this may be regarded as evidence of permeability to ions. It is, however, quite possible that in these experiments the cells are readily permeable to ions only because they are abnormal. It is well known that plasmolysis produces injury and that injury is accompanied by changes in permeability. It is also possible that the cells may subsequently recover from such injury and appear to be normal; in this case the permeability to ions would be only a temporary one. Injury might affect only a portion of the cell surface (possibly numerous small areas). Experiments on large multinucleate cells (*Valonia*, *Nitella*, *Caulerpa*, *Bryopsis*) indicate that a portion of the cell surface may be greatly altered while the remainder remains in normal condition for a long time afterward.

If recovery from plasmolysis in salt solutions depends on alterations of permeability we should expect the rate of recovery from plasmolysis to correspond somewhat with the amount of alteration. If the alteration goes too

far the cell may become so permeable that no recovery is possible, but up to a certain point increase in permeability would increase the rate of recovery from plasmolysis if exosmosis were not greater than endosmosis. From this standpoint we might expect the recovery in sodium chloride to be more rapid than in a balanced solution of sodium and calcium chlorides (or in sea water) since alterations in permeability would be more rapid in sodium chloride. This seems to be the case. When recovery occurs in balanced solutions it is possible that it is also due to alterations in permeability, since it is well known that hypertonic balanced solutions may cause injury.

If ions are unable to penetrate normal protoplasm how are we to regard the experiments which indicate that marine plants bathed in sea water allow ions to enter the protoplasm and thus conduct the electric current under conditions which seem to ensure that the cells are in a normal state?

In the first place it is possible that if the cell normally opposes a high resistance to the

passage of ions this resistance may be overcome under electric stress so that ions may be forced through the surface of the protoplasm, although they would not enter if the electric potential were absent. In this case the measurement of electrical conductivity would reveal changes in resistance to the passage of ions brought about by various conditions, but the passage of the electric current would not mean that ions could penetrate to an appreciable extent in the absence of an applied potential. From this standpoint we may say that the general conclusions derived from electrical experiments would not be changed except that the normal cell would not be regarded as permeable to ions. The measurement of changes in resistance to the passage of ions brought about by abnormal conditions and the conclusions drawn from these measurements would still be valid.

In the second place it is possible that the measurements of conductivity do not indicate the passage of ions through the protoplasm, as has been supposed. If the cell acts as a condenser an alternating current may pass without actual

transfer of ions through the protoplasm as indicated by some older experiments and more recently made highly probable by investigations carried out in my laboratory by Mr. L. R. Blinks. In that case the increase in conductivity (as measured by the alternating current) which occurs when a cell is injured may be regarded as analogous to the change by which a condenser becomes a conductor. If the cell surface is covered with a non-conducting substance injury might alter this substance in certain places, so that the conductivity would increase. If this view should turn out to be correct we should nevertheless continue to regard the measurements of the electrical conductivity of living tissues by means of the alternating current as of great value in detecting changes of permeability. The reasons for this have been given in discussing the electrical method.

If we adopt the hypothesis that ions can enter normal protoplasm only very slowly it is evident that injury and death are accompanied by increased permeability to ions. There is good evidence that this is the case.

Although undissociated molecules appear to enter much more rapidly than ions it is not to be expected that all undissociated molecules enter the protoplasm with the same readiness and in many cases it appears as though they cannot penetrate into the sap unless they can combine chemically with some constituent of the protoplasm.

Some such considerations may possibly explain the excluding mechanism whereby, for example, *Valonia macrophysa* prevents more or less completely the entrance of undissociated molecules of such substances as phenol, glucose, lactose, sucrose, and many others, while admitting more or less freely undissociated molecules of hydrogen sulphide, carbon dioxide, ammonia, ethyl alcohol, dimethyl-glyoxime, paraphenylene-diamine, and urea.

It would therefore seem as if the surface forms a barrier which more or less completely prevents the passage of ions, and as if undissociated molecules can enter the sap only by passing through a layer which excludes such sub-

stances as are insoluble in it or unable to unite with it chemically.

In this connection we may recall the fact that a monomolecular film on the surface of water does not prevent the evaporation of water. It is evident that water molecules pass through the oil. In this case the escape of water may not be governed by the solubility of the water in the oil but by the size and speed of the water molecules and the direction in which they strike the surface. If we extend this idea to other kinds of molecules we must also consider that the shape of the molecules may be of importance in some cases. It is of course quite possible that the surface of the protoplasm is a non-aqueous monomolecular layer.

Let us now endeavor to picture a mechanism by which certain substances may accumulate in the cell. Suppose that undissociated molecules of a weak base penetrate until their concentration is the same inside and outside (the acidity and other conditions being the same inside and outside). If now the cell produces acid, some of the molecules of the weak base will dissociate and

more will diffuse in until the concentration of undissociated molecules again becomes the same within and without. This process may continue until the inside concentration of total weak base (ionized plus non-ionized) becomes much greater inside than outside. A weak acid would accumulate in the cell in the same way if the cell produced alkali. We may extend this hypothesis to include not only weak acids and bases but all other substances, organic or inorganic, which are able to change with changes in the concentration of hydrogen ions. Such changes (including tautomerism, formation of complex salts, hydration, hydrolysis, etc.) may affect substances to a greater extent than is at present suspected.

It may seem possible in some cases that accumulation may be due to a Donnan equilibrium. This exists when one or more non-diffusible ions are present in the cell or in the external solution and it requires that the following ratio should exist for any pair of diffusible ions:

$$\frac{\text{conc. cation inside}}{\text{conc. cation outside}} = \frac{\text{conc. anion outside}}{\text{conc. anion inside}}$$

If we have ten times as many hydrogen ions inside as outside and if potassium ions are present inside we should expect ten times as many potassium ions inside as outside providing both these cations are diffusible. This might seem at first sight to afford a means of explaining the accumulation of potassium when the sap is more acid than the external solution, as is normally the case with *Nitella* and *Valonia*. But it also requires that the accumulation of all other diffusible cations should take place to the same extent and this is not the case.

We see, therefore, that we may explain accumulation in many cases where a substance exists in two forms whose relative proportions vary with the differences in acidity, and it is possible that this explanation applies to more substances than is at present suspected. It may be objected that this would require that all cells having equally acid sap should behave in the same way in regard to accumulation. This, however, would not necessarily be true, since differences in permeability may bring about differences in the relative proportions of electrolyte in the sap.

Although the protoplasm might not change the final equilibrium it might prevent a true equilibrium being reached by causing some substances to enter so slowly that no true equilibrium would be reached during the period of growth.

The problem is a very complicated one and it seems clear that we cannot hope to attack it successfully without the help of careful quantitative work. The studies here described are a preliminary attempt in this direction.

These studies lead me to look upon the semipermeable membranes of the cell as even more important than I supposed. In certain cases it seems quite possible that in a cell immediately after death the same substances may be present and the same reactions go on as just before it was killed. But the difference between the living and the dead state is very marked in respect to the semipermeable surfaces: after death they lose their selective power and the internal and external solutions begin to mingle. When the semipermeable surfaces no longer safeguard the privacy of the chemical processes of the cell the vital activities are unable to continue in normal

fashion. The difference between a cell immediately before and just after death might therefore be essentially a difference in the structure and chemical composition of the semipermeable surface.

It may be added that such semipermeable surfaces are not confined to the exterior of the cell but exist also at the boundaries of nuclei, plastids, microsomes, and other structures in the cell. It may be that the chief advantages of cell division (as well as of the differentiation of cell organs) is to provide such surfaces and thereby to segregate various vital activities.

In this connection we may recall that Loeb has insisted that the colloidal properties of matter are manifested only where semipermeable surfaces exist. A study of such surfaces is important for chemistry as well as for biology and it is possible that further investigations in this field will lead to contributions to chemical theory just as did the work of Pfeffer at an earlier period.

These and other studies now in progress have changed our point of view and are bringing a

wealth of fresh problems. Although some of these may be insoluble in the present state of science, we ought at least to see how far we can go. The whole subject is so important that we can afford to neglect nothing which may help us to understand the mechanism of these fundamental activities of the cell.

Since this lecture was given additional facts have come to light which have necessitated some changes in viewpoint.

The investigations of Blinks and Howe indicate that what is here called *Valonia ventricosa* is in reality a species of *Halicystis*, which puts a different face upon a part of the discussion. The accumulation of dye appears to be due to its behavior as a weak base which enters the sap and dissociates rather than to its combination with the proteins of the sap (*cf.* Irwin, M., *J. Gen. Physiol.*, 1926-1927, x, 75). It seems possible that the accumulation of potassium can be explained by assuming that it enters as potassium hydrate, is converted to potassium carbonate and

bicarbonate, and becomes potassium chloride by exchange of the carbonate and bicarbonate ions produced inside the cell for chloride ions coming in from the sea water. The distribution of chloride ions would be determined in this case by the Donnan principle (*cf.* Osterhout, W. J. V., *Proc. Soc. Exper. Biol. and Med.*, 1926, xxiv, Dec. No.).

The fact that the electrical resistance of the protoplasm is so high might be explained, as stated above, by supposing that the outer layer of the protoplasm consists of a layer which is only slightly permeable to ions and which acts to a considerable extent as a condenser. Later investigations by Dr. Blinks indicate that (due to the polarization capacity) much the same effect would be produced if ions of one sign entered the protoplasm much more readily than those with the opposite charge. Further investigation will be needed in order to clear up this question.

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